

## DIENTAMOEBEA FRAGILIS: SOME FURTHER OBSERVATIONS

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In 1935 (1) the writer reported *Dientamoeba fragilis* as a probable pathogen and recorded some observations upon the identification and viability of this amoeba. Since then 37 cases of *D. fragilis* infection have been encountered and additional information obtained on various features of this amoeba and its relation to the human host.

### INCIDENCE

For a general review of the literature dealing with the incidence of *D. fragilis* the reader is referred to Brug (2).

In Panama and the Canal Zone its presence was first reported in 1927 by James (3) who had then, he states, encountered it twice. Hegner, Johnson, and Stabler (4) in their survey of 156 Panamanians living in villages a few miles from Panama City and 20 Rio Chico Indians, found *D. fragilis* once in the former group. They examined, however, only one direct smear from each fecal sample. Faust (5) examined a series of more than 2,000 persons from Panama and the Canal Zone and Anderson (6) 1,000 patients in Santo Thomas Hospital, Panama, but the reports on these surveys consider only *Endamoeba histolytica* and give no data on *D. fragilis* or on any of the other intestinal protozoa.

Although we have made no direct attempt to determine the incidence of *D. fragilis*, some data on two conditions bearing upon the prevalence of this amoeba have been obtained. One

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concerns the occurrence of new infections among inmates of an institution for the insane, and the other the occurrence within families residing in Panama and the Canal Zone.

(1) *The occurrence of new infections.* In a series of 38 adult inmates, 28 men and 10 women, in Retiro Matías Hernández<sup>2</sup> who had been treated with carbarsone for amoebiasis, 16 *D. fragilis* infections, 13 in men and 3 in women, were disclosed during a follow-up period of one year. These patients had shown no *D. fragilis* in five to ten fecal samples examined prior to this treatment and had received four to eight times as much carbarsone as the amount which in six cases apparently has eradicated *D. fragilis* (see discussion of treatment). It therefore appears practically certain that none in this group was the host of *D. fragilis* after completion of the carbarsone treatment. The 16 infections which appeared during the year among these 38 individuals were then new infections representing an annual rate of 42.1 per cent. The earliest infection was discovered on the fifteenth day after completion of the treatment; the others appeared almost equally distributed among the twelve months.

To evaluate these figures one needs to know how these patients were examined and what the chances of detecting *D. fragilis* were. All patients were examined according to the following schedule: Three normally passed stools and one stool following catharsis during the first week after completion of the treatment; one normally passed stool each week during the following ten weeks; three normally passed stools and one following catharsis during the twelfth week, and then one normally passed stool monthly during the remaining nine months of the year. In addition, patients who passed several firm dry stools in succession were examined after catharsis. In all 1,204 fecal specimens from the 38 patients were examined during the year; 239 of these were formed and too dry to permit the existence of *D. fragilis* and other vegetative protozoan forms and since we have identified no cysts of *D. fragilis* these stools were useless for the purpose of detecting this amoeba. There remained then 965 stools, 25.3 per individual, of suitable consistency (liquid,

<sup>2</sup> An institution for the insane of the Republic of Panama.

mushy, or soft formed) for the detection of *D. fragilis*. Each stool was examined in two to four wet smears and in wet-fixed preparations. The latter were stained with Heidenhain's iron-hematoxylin and searched for at least twenty minutes using a 2 mm. oil immersion objective and  $\times 6$  ocular. All wet smears were examined by the writer; the fixed smears were stained and examined by a skillful and dependable technician with proved ability in the identification of intestinal protozoa. Stained smears which presented doubtful findings were also examined by the writer.

It is probable that these methods were adequate for the detection of *D. fragilis* infections except those with a short duration which may have run their course in intervals between the examinations of suitable fecal samples (see discussion of duration of infection). The actual number of infections may thus have been slightly higher.

What the incidence is at any given time among the various groups of inmates in Retiro Matias Hernández is not known but it appears likely that it may exceed the rate of 36 per cent found by Brug (7) among asylum inmates in Holland and even equal the rate of 51.4 per cent found by Svensson and Linders (8) in an asylum for mental cases in Sweden.

(2) *The occurrence within families.* A Panamanian technician in this laboratory who had been found to be a carrier of *E. histolytica* requested that his whole family be examined. The family consisted of nine members, all adults, and lived in a small house in the city of Panama. The following infections were discovered: *E. histolytica* 2, *E. coli* 3, *Endolimax nana* 1, *Iodamoeba bütschlii* 1, and *D. fragilis* 4. Only three fecal samples from each member were examined and of these less than one-half were of a consistency suitable for *D. fragilis*. A more extended search could therefore have been expected to reveal not only a higher total incidence of protozoa but also a relatively higher incidence of *D. fragilis*.

A white family consisting of the parents and two children, age five and seven years, living in the Canal Zone requested examination for intestinal worms. Only one stool was obtained from

each individual. All four stools were mushy in consistency and all contained an abundance of *D. fragilis*. The father also had *E. coli* and *I. butschlii*.

These findings seem to indicate that *D. fragilis* has little difficulty, probably less than the other intestinal amoebae, in finding new hosts. However, that it does not always infect exposed individuals with facility is demonstrated in one of our records, as follows: A Panamanian laboratory technician and his wife were treated for amoebiasis and the result checked by weekly or more frequent stool examination. On the thirty-fifth day after completion of the treatment the wife was found to have *D. fragilis*. Since then, now a period of five months, her stools whenever examined have shown numerous *D. fragilis* except a few times when they were formed and dry. During this time all fecal samples from the husband were negative except on the seventy-fifth day after the appearance of the infection in the wife when a mushy stool showed a few degenerated *D. fragilis*. The long period of exposure without infection and the very transient character of the infection when finally acquired suggest that the husband is immune to *D. fragilis*, or, perhaps, merely that his intestinal contents is persistently unfavorable for the growth of this amoeba.

#### DURATION OF INFECTION

The duration of an intestinal protozoan infection cannot, it would seem, be determined accurately. Even if daily or all stools be obtained and examined it seems likely that an infection may be present for some days without being detected and similarly that it may persist for some time after our methods fail to find the particular protozoon in the fecal material.

Obviously the schedule of examination for the 38 inmates referred to above did not delimit the duration of the 16 *D. fragilis* infections very accurately. The records show that some infections might well have been present for weeks or even months prior to their discovery. However, it was rather definitely determined that not less than seven of the sixteen infections disappeared during the year. Before an infection was considered as

having been lost, at least ten negative stools of a mushy consistency including two produced by catharsis were obtained and examined thoroughly in two to four wet smears, in wet-fixed stained smears, and by cultures.

Among cases not included in this series the following data on duration may be recorded. From one individual only a single positive stool was obtained. During the month preceding this examination, four mushy stools including one after catharsis had been examined and found negative for all protozoa, the last of these negative samples having been obtained a week prior to the positive stool. During the week subsequent to the one appearance of *D. fragilis* no stools were examined but after that, numerous mushy, liquid, and post-catharsis stools have been examined over a period of five months and found negative. These observations should limit this infection to two weeks. Several infections have been current for a year. One patient carried the infection for a year and a half (553 days) and then lost it without treatment. He was examined regularly once a week and at many odd times and practically every stool of suitable consistency showed *D. fragilis* except during an attack of acute entero-colitis, probably bacillary dysentery, which lasted about ten days. No *D. fragilis* could be found in the pink purulent exudate of this disease, but as soon as mushy fecal matter appeared in the stools *D. fragilis* was again present.

#### DIAGNOSIS

The author has recorded previously (1) that *D. fragilis* can be identified rather easily not only in wet-fixed stained preparations but also in wet smears, and that, contrary to what had been generally believed, it is not a fragile amoeba which quickly disintegrates after leaving its host but that it is quite hardy, able to survive for 24 to 48 hours in the evacuated feces. Wenrich (9) has reported a similar observation on the viability of *D. fragilis* having found in one stool that considerable numbers of the amoebae remained after 24 hours and that after 48 hours there were still a few survivors. More recently Brug (7) obtained positives cultures from stools 6½ to 13½ hours old but not from

stools 20 or more hours old. However, he examined only a few stools and some of these were emulsified in tap water which undoubtedly destroyed the *Dientamoeba* in a few minutes.

In wet smears *D. fragilis* presents three extraordinarily distinctive features, two of which are displayed in normal saline smears and one in aqueous smears. In the former, when at rest, the faultless circular outline, and when active, the film-like pseudopodia with sharp points; in the latter, an explosive rupture of the ectoplasm with complete evacuation of the endoplasm, followed by restoration of a spherical ectoplasmic shell.

When first seen in normal saline smears *D. fragilis* is at rest and perfectly circular in optical section. This period of "paralysis" varies from a minute to an hour or more. Even when the smear is made immediately after evacuation of the stool and chilling avoided, motility is delayed. (Only once has immediate motion been observed; it occurred in a smear from a freshly passed stool with a shower of unusually active *Dientamoebae*.) The experienced observer will recognize the fresh *D. fragilis* even in this stage of immotility depending then upon the circular outline and the rather characteristically thin and finely granular endoplasm. When vacuoles of degeneration appear, *Dientamoebae* before regaining motility may closely resemble rounded up, vacuolated forms of the other intestinal amoebae and unless occurring in great numbers the identity should be no more than suspected until pseudopodia appear.

The first sign of motility is merely a change in the contour, frequently to a pear-shaped outline. A part of the ectoplasm then flattens out into an extremely thin film the edge of which sometimes can be discerned only with favorable light and careful focusing. This edge is constantly changing and sooner or later the characteristic sharp points or corners appear.

The persistence of pseudopodial energy in some specimens is remarkable. Though the endoplasm may be crowded with vacuoles or degenerated, pseudopodial movements often appear after a period of time.<sup>3</sup> To save time and patience when dealing

<sup>3</sup> It is possible that Jepps and Dobell (10) failed to recognize this delayed motility when they concluded that *Dientamoebae* "rapidly cease to move and die."

with such devitalized Dientamoebae it is well to set the smears aside for one-half hour or more until motility is recovered. In old fecal samples and sometimes even in the freshly passed stools their vitality may be so impaired that no motility or merely a slight change in contour occurs. However, these degenerated Dientamoebae usually retain the toughness and resiliency of the ectoplasm and present the diagnostic reaction in aqueous smears.

The use of aqueous smears as an aid in the identification of intestinal amoebae (11) requires considerable experience except in the case of *D. fragilis*. The diagnostic manner of its destruction in this preparation is an event which one quickly can learn to recognize. For the convenience of the reader a description of this phenomena will again be given. The aqueous smear in which water is used instead of normal saline should be made rather thin. By the time the smear has been prepared and placed under the microscope the Dientamoebae have begun to distend. As the osmosis progresses the endoplasm becomes thin and lustreless, and quite transparent. The various inclusions become clearly outlined and the nuclei visible as slightly refractile discs. After one to ten minutes the distention terminates with an explosive rupture of the ectoplasm and expulsion of the contents following which the ectoplasmic shell quickly regains its faultless spherical form. Within this shell there always remain some minute particles in brownian movements, and sometimes the nucleus, or nuclei, in the case of binucleate individuals. The evacuated mass quickly disappears but the ectoplasmic shell usually remains intact for several minutes, even up to 15 minutes. Occasionally some individuals, rarely all, rupture with immediate destruction of the ectoplasm as well as the contents, but even this explosive destruction can readily be recognized as diagnostic of *D. fragilis*. Before rupturing, *D. fragilis* may resemble other protozoa distending in aqueous smears: *Chilomastix mesnili* trophozoites and *Trichomonas hominis* present the same thin contents, and the nuclei in the distending *E. nana* and *I. butschlii* have the same disc-like appearance, but these protozoa instead of rupturing with evacuation of the endoplasm merely crumple into a wrinkled mass of debris. *E. histolytica* and *E. coli* may

rupture rather explosively with extrusion of a small part of the contents and sometimes the nucleus, but there never is a complete emptying with the ectoplasmic shell springing back to its spherical form like a rubber ball, as in *D. fragilis*.

When there are very few *D. fragilis*, for example one or two per smear, or when many distending blastocysts, *C. mesnili* trophozoites, or *T. hominis* are present, the aqueous smear may fail on account of the short time available for the search. However, this difficulty will not often be met with after the examiner learns to spot the distending *Dientamoeba* under low power (4 mm. objective and  $\times 6$  ocular).

By this lengthy discussion of the diagnostic possibilities of wet smears the writer has not meant to give an undue importance to these preparations as compared with wet-fixed stained smears. The latter are necessary for checking doubtful findings in wet smears and as permanent records of important findings. Their relative value for detecting *D. fragilis* infections will depend to a great extent upon the particular experience and the skill of the individual worker. The records upon which this report is based favors slightly the wet smears. Of 277 fecal samples which were found to be positive for *D. fragilis*, 163 were examined in both wet smears and wet-fixed stained preparations. Of these, 112 were positive in both preparations, 33 in only the wet smears and 18 in only the stained smears. Thus 145 of these infections, 88.9 per cent, were detected in the wet smears and 130, 79.7 per cent, in the stained smears. All of the 51 stools which were found positive by only one of the two methods contained very few *D. fragilis*, one or two per smear. The higher scoring of wet smears under these conditions could be expected since our routine permitted a larger volume of feces to be searched in the wet smears. This routine included three or four wet smears under 22 x 22 mm. coverglasses, the examination of which required from ten to fifteen minutes, and one stained smear searched for twenty minutes which search usually covered less than the area of one wet smear. Considering then that less time was used for the examination of wet smears and that in spite of this they revealed more of the infections it would seem that

within a given time the examination of a fecal sample by wet smears was more efficient for finding *D. fragilis* than examination by wet-fixed stained smears.

The fact that *D. fragilis* is readily identified does not imply that the diagnosis of the infection always is an easy procedure. Not infrequently this amoeba, like the other intestinal protozoa, becomes very rare even in the most suitable stools and a number of smears may have to be searched before a single individual can be found. Occasionally several consecutive stools which grossly may appear to be ideal for Dientamoebae have proved negative in spite of a most determined search. In general, however, the frequency of occurrence of *D. fragilis* in fecal samples of infected persons has been seen to depend mainly upon the consistency of the stools. The mushy stool passed normally or after a mild laxative has been the most suitable. Purgation with watery evacuations usually have been quite unsatisfactory. In flourishing infections even the soft formed stools have shown *D. fragilis* quite commonly and occasionally a few survivors have been found in formed firm stools.

It was these observations that led us to examine not less than ten stools of suitable consistency before considering an infection spontaneously lost.

#### SYMPTOMS AND CLINICAL FINDINGS

No thorough clinical study was made of any of the cases involved in this report. A clinical history with particular reference to gastrointestinal symptoms was obtained from twelve cases. None of these had the rather severe gastrointestinal disturbance previously reported by the author (1), but six complained of some abdominal distress at times, such as mild cramps with gas and rumbling in the bowels and of mushy stools with burning in the rectum after defecation. Obviously it is quite possible that these rather common symptoms had no relation to the *D. fragilis* infections. However, of six of these patients who received treatment resulting in eradication of *D. fragilis*, four were relieved and felt improved in their general health.

## TREATMENT

In the case reported by the writer in 1935 *D. fragilis* apparently was eradicated by four doses of carbarsone, each 0.25 gram, taken during a period of 24 hours. Since then 6 more cases have been treated with carbarsone.

The first 4 cases were members of the Panamanian family discussed under incidence. They were adult women weighing from 109 to 124 pounds. Each received carbarsone by mouth, 0.50 gram twice daily after meals for two days, a total of 2 grams. Prior to this treatment they had been observed for one to four weeks during which time five to eleven stools were found positive for *D. fragilis*. A mushy stool was obtained from each case on the day preceding the treatment and found to contain large numbers of *D. fragilis*. Subsequent to the treatment no *D. fragilis* have been found in these cases although searched for in numerous stools for a period of one year.

Case 5 was a white adult male American, weight 152 pounds. After having carried the infection for four months during which the stools showed a flourishing growth of *D. fragilis* he was given the same amount of carbarsone, 2 grams in two days. Since the treatment ten stools of a mushy consistency suitable for *D. fragilis*, four of these after catharsis, have been obtained in a period of two months and found negative.

Case 6, a white adult male American weighing 132 pounds received three courses of carbarsone, each 2 grams in two days. Prior to the first course his stools had been examined daily for 78 days and had shown *D. fragilis* in large numbers except in a few formed dry samples. After this first treatment the stools were negative for twenty-one days, then *D. fragilis* reappeared and was present in suitable stools until the second course was given, thirty-five days after the first. This time there were negative stools for only six days. It was known by this time that at least one of the patient's children also carried a *D. fragilis* infection and it appeared possible that reinfections instead of recurrences had occurred. No further treatment was therefore given until nine months later when all members of his family left for an extended vacation. At this time the patient's stool

still showed a luxurious growth of *D. fragilis*, and he was given the third course. Since then no *D. fragilis* have been found although numerous mushy stools, some passed after catharsis, have been examined during a period of two months.

Although it is quite possible that in the last case the first two courses of carbarsone failed to eradicate the infection, the success of the other six treatments suggests that carbarsone even in the small total amount of 2 grams will prove to be an effective means of eradicating *D. fragilis* infections.

#### ABSENCE OF CYSTS

We have made many efforts to find *D. fragilis* in an encysted stage but have failed. Forms of trophozoites similar to the cysts reported by Kofoid (12) and forms resembling the "pseudocysts" described by Wenrich (9) have been seen at times in our stained preparations. It is well to recall in this connection that in stained films some trophozoites of any of the intestinal amoebae may very closely resemble the corresponding cysts unless the nuclear system has developed to a stage which is characteristic of the cysts. The cysts described by Kofoid have the same nuclear system as trophozoites and their cytoplasm and vacuoles, as well as the minute inclusions considered chroma-toidal may well belong to the vegetative forms. Similarly the cyst walls which according to Kofoid<sup>4</sup> are very faint and difficult to demonstrate may merely be the ectoplasm of a rounded up trophozoite.

There is one readily demonstrable characteristic of all the known cysts of the intestinal protozoa, namely a cyst wall which withstands the osmotic pressure of water, making it possible for the cysts to remain in an aqueous medium unharmed for days. It seems reasonable to assume that the cysts of *D. fragilis*, if existent, would be similarly constructed. We have been un-

<sup>4</sup> Personal communication, 1937, as follows: "We have occasionally found cyst walls on *Dientamoeba fragilis* in stool smears, but never as well developed as in other cysts of intestinal amoebae. The walls do not stain with the usual staining method and are very faint and difficult to demonstrate. They apparently are much thinner and less firmly formed than in other intestinal amoebic cysts. We have not identified cysts of this amoeba from laboratory cultures."

able to find any such forms although they have been searched for extensively. More than a thousand aqueous smears from fecal samples of thirty-seven individuals infected with *D. fragilis* have been examined and have failed to show any bodies that possibly could have been cysts of *D. fragilis*. Similarly negative were concentrations of 168 stools from 17 persons who at the time of collection of these stools were known to be carriers of *D. fragilis*. Of these 168 stools, 120 were formed and of a suitable consistency for the cysts of, at least, the other intestinal amoebae. The remaining 48 stools were mushy and contained a few *D. fragilis* trophozoites; they were concentrated with the possibility in mind that cysts of *D. fragilis* would be passed in the softer stools. The concentrations were made by the sugar flotation method described by Yorke and Adams (16), a method which in our hands has been found very satisfactory for concentration of cysts of intestinal protozoa.

Six of these concentrates were also cultured under the assumption that cysts of *D. fragilis* might be present although not identified by microscopical examination and that they might excyst *in vitro* and develop a growth. The Dobell-Laidlaw medium with rice-starch and acriflavine 1:20,000, as recommended by Svensson and Linders (8), was used. The cultures were examined daily for six days. No amoebae developed. In control cultures inoculated from positive fecal material before concentration, *D. fragilis* grew luxuriantly.

#### SUMMARY

Some observations upon the incidence, duration of infection, diagnosis, clinical findings, and treatment of *D. fragilis* infections have been presented, as well as a report of some attempts to find encysted forms.

*Incidence.* In a group of thirty-eight inmates of an insane asylum, sixteen new infections with *D. fragilis* occurred during the course of one year, an annual rate of 42.1 per cent. Two instances of familial infection are recorded, in a Panamanian family of nine members four had *D. fragilis*, and in a white

family of four including two children less than ten years of age, all had the infection.

*Duration of infection.* It was found that some *D. fragilis* infections apparently terminated spontaneously. The shortest period to which our methods of examination limited any infection was two weeks. One infection, the presence of which was checked at least once a week, lasted a year and a half. Several infections have been observed for a year and are still current.

*Diagnosis.* *D. fragilis* has been found to be the most readily recognized trophozoite of the intestinal amoebae. Not only can it be identified positively on its characteristic nuclear system in wet-fixed stained preparations but even in unstained wet smears it presents diagnostic features which are so constant that identification is practically always possible. Mushy stools passed normally or after mild laxatives contain the amoeba most frequently and in the largest numbers. In some infections *D. fragilis* at times becomes very rare even in these stools and its detection may then be a difficult task.

*Clinical findings.* Clinical histories were obtained from 12 cases. Six of these complained of some intestinal distress which, however, may not have been caused by the *D. fragilis* infection.

*Treatment.* Six adults were treated with carbarsone, 0.50 gram twice daily for two days, a total of 2 grams. In five of these, *D. fragilis* apparently was eradicated by one course. The sixth case received three courses before the infection disappeared. There was a probability that in this case reinfections rather than recurrences occurred after the first two courses.

*The absence of cysts.* Having recognized the possibility that in stained smears from fecal samples some trophozoites may resemble and be mistaken for cysts our search for encysted forms of *D. fragilis* was made after the trophozoites had been destroyed in water. More than a thousand aqueous smears and 168 concentrations made from stools of individuals who carried the infection failed to reveal any bodies that possibly could have been cysts of *D. fragilis*. Cultures from six concentrates were also negative.

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